Detection of organic anions in water through complexation enhanced fluorescence of a macrobicyclic tris-acridine cryptand

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The water-soluble macrobicyclic cryptand 1 containing three fluorogenic acridine units has been synthesized and its photophysical and substrate binding properties have been examined. The free hexaprotonated compound 1-6H$^+$ displays dual (monomer/excimer) fluorescence with a particularly low quantum yield ($\Phi_F = 10^{-3}$) in comparison with that of the related monochromophoric compound 2 ($\Phi_F = 6.8 \times 10^{-2}$). Its fluorescence is strongly affected on complexation with various organic anions (carboxylates, sulfonates, phosphates), *i.e.* a strong increase of the monomer band is observed with concomitant disappearance of the excimer contribution. The fluorescence revival of 1, which arises from both conformational changes and specific electronic interactions with the substrate, allowed the determination of high stability constants for the 1:1 complexes (K_s ranging from 10^3 to 10^7 dm^3 mol^{-1}). The magnitude of this effect has been correlated in terms of relative fluorescence quantum yields (Φ_R) to the selectivities observed (polyanion/monoanion; aromatic/aliphatic; chain length selectivity) and to the factors involved in the strength of the association (electrostatic and hydrophobic forces; structural complementarity). In the case of the naphthalene derivatives, the occurrence of a donor–acceptor energy transfer process demonstrates the formation of an association where the substrate and the receptor are in close proximity. Finally 1-6H$^+$ binds tightly to mono- and oligo-nucleotides and moreover appears able to discriminate between homopyrimidine and homopurine sequences by its light-sensitive response.

The bis-tren macrobicyclic structures obtained from the condensation of tris(2-aminoethyl)amine (tren) with various dialdehydes[1] are well adapted to the strong complexation of a wide range of substrates: in their neutral imino or amino forms they behave as binucleating ligands for transition metal cations[2] and in their polyammonium forms they have been shown to trap inorganic, as well as organic, anions.[3]

Due to the biological importance of the complexation by enzymes of negatively charged species (*i.e.* carboxylates and phosphates), we have been interested for years in the design of anion receptors which could mimic biological activities such as anion recognition, transport and catalysis.[4] Thus we previously described the formation of inclusion complexes between a tris-biphenyl bis-tren cryptand and the terephthalate dianion,[3b] which unambiguously demonstrates the possibility of encapsulating an aromatic anionic guest in such an ellipsoidal molecule.

In the course of our study we synthesized the novel cryptand 1 (Fig. 1) in which three fluorogenic acridine units have been incorporated and we describe herein its complexation and spectroscopic properties.

The presence in the molecule of the three acridine rings held together in close proximity, gives compound 1 remarkable structural and photophysical properties which distinguish it from related bis-tren macrobicycles in the following ways: (*i*) the three condensed aromatic units constitute a hydrophobic microenvironment maximising van der Waals interactions; (*ii*) the highly lipophilic character of the core is balanced by the two protonated tren moieties providing a good solubility in water (pH 6) and an efficient electroattracting system capable of bringing about complex formation with anionic guests; and (*iii*) the free hexaprotonated receptor 1-6H$^+$ exhibits a very weak fluorescence mainly due to the short interchromophoric distance highly favourable to the occurrence of intramolecular interactions between the three acridine moieties. By contrast the addition of various anionic substrates instantaneously results in a dramatic enhancement of the fluorescence quantum yield of 1 by factors of up to 27.

The recognition of cations through photophysical changes of various receptors has been extensively investigated.[5] On the other hand examples of optical detection of anion binding through fluorescent complexing molecules are still scarce,[4b,d,e,6] largely confined to inorganic ions, and rely frequently on the inherent quenching properties of the anion. We therefore decided to examine the ability of various anionic substrates to affect the fluorescence of the protonated receptor 1-6H$^+$ on formation of stable complexes.

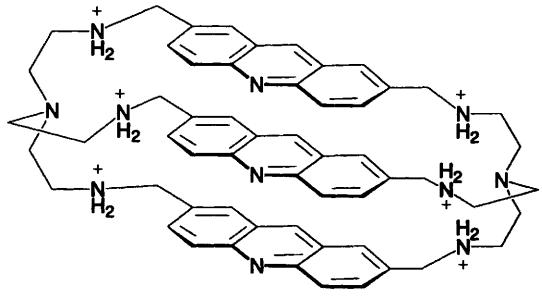


Fig. 1 Schematic representation of the hexaprotonated form 1-6H$^+$ of the receptor 1 at pH 6

Spectroscopic properties of 1

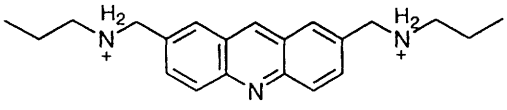
The macrobicyclic compound 1 was obtained from the well known strategy[1,2a] involving the (2 + 3) condensation of tren and acridine-2,7-dicarbaldehyde,[4e] followed by treatment with $NaBH_4$ and recrystallization in HCl (1 mol dm^{-3}). The characteristic pH dependance of the absorbance of 1 allows the evaluation of the pK_a value of the acridine units. When the pH is lowered from 6 to 2, a continuous increase of the intensity of the band lying near the visible (λ_{max} 360 nm) is observed with a small blue shift of the maximum wavelength; this increase is especially steep between pH 4 and 2 [pH 6, ε(360 nm) = 14 300; pH 4, ε(360 nm) = 18 000; pH 2, ε(356 nm) = 42 000†] and is

† ε Values are given in units of dm^3 mol^{-1} cm^{-1}.

Table 1 Comparison of the molar extinction coefficients (ε) and of the quantum yields (Φ_F) of receptor **1** and monomer **2** measured in 1 mmol dm^{-3} cacodylate buffer (pH 6, 4 mmol dm^{-3} NaCl, 20 °C)

Compound	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}	Φ_F
1	360	14 300	0.001
2	359	14 360	0.068

accompanied by the formation of an isosbestic point at 321 nm. Furthermore the concomitant appearance of a shoulder of weaker intensity at 401 nm (pH 2, ε = 8500,) which is characteristic of the presence of the acridinium cation [7] is noted. These spectral perturbations are well kown to be representative of the acid–base equilibrium between the cationic and neutral forms of the acridine nuclei.[4e,7] Similar variations of the UV–VIS absorption spectrum have been reported for the monomeric reference compound **2** and for related dimeric



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acridines,[4e] and were in accordance with the pH-metric determination of the pK_a of the ring nitrogen which was found to be 3.65. Thus a low pK_a value (< 4) seems to characterize the acridine nuclei substituted by protonated aminomethyl groups in the 2,7 positions, whereas acridine itself has a pK_a of 5.6.

On the basis of these results and of the acidity constants of tren and bis-tren compounds,[3a] we may expect that species bearing neutral acridine units and hexaprotonated or pentaprotonated tren linkers (**1-6H**$^+$, **1-5H**$^+$) predominate at pH 6.

A comparison of the molar extinction coefficient values of **1** and of reference compound **2** shows a large hypochromism of the π–π^* transition with ε(**1**) $\ll$ 3 × ε(**2**), but no bathochromic shift is observed (Table 1). These features indicate that there is no significant interchromophoric electronic effect and reflect the existence of some weak intramolecular interactions between the three acridine rings in the ground state as reported for bis-chromophoric systems.[8]

The fluorescence emission spectrum of **1** is extremely weak compared to that of the monomer **2** [Fig. 2(*a*)], as shown by the low quantum yield measured for **1** compared to that of **2** (Table 1). Despite the low intensity, two bands can be characterized, at 460 nm, attributed to monomer-like emission somewhat red shifted, and farther to the red at 555 nm, a structureless band ascribable to an excimer. The red shift of the monomer band already observed in macrocyclic bis-acridines[4e] may be interpreted by the creation of a hydrophobic environment close to the chromophores. The excimer character of the second band agrees with various observations: indeed the excitation spectra scanned on the monomer (460 nm) and on the excimer wavelengths (555 nm) were found to be similar, pointing to the common origin of the two emissions, and the intensity ratio of the two bands is not altered by a change in the excitation wavelength. The spectrum and the maximum of the excimer emission can be visualized from the difference between the spectra displayed by **1** and **2** [Fig. 2(*b*)] and are comparable to those of acridine excimers identified in aqueous solution of 9-aminoacridine.[9]

In accordance with the UV–VIS results, these findings confirm that the low quantum yield of **1** relies mainly on the occurrence of intramolecular interactions in the excited state and reflects the close proximity of the acridine rings as depicted by the excimer band.[8] The contribution of a quenching effect *via* photoinduced electron transfer from deprotonated benzylic

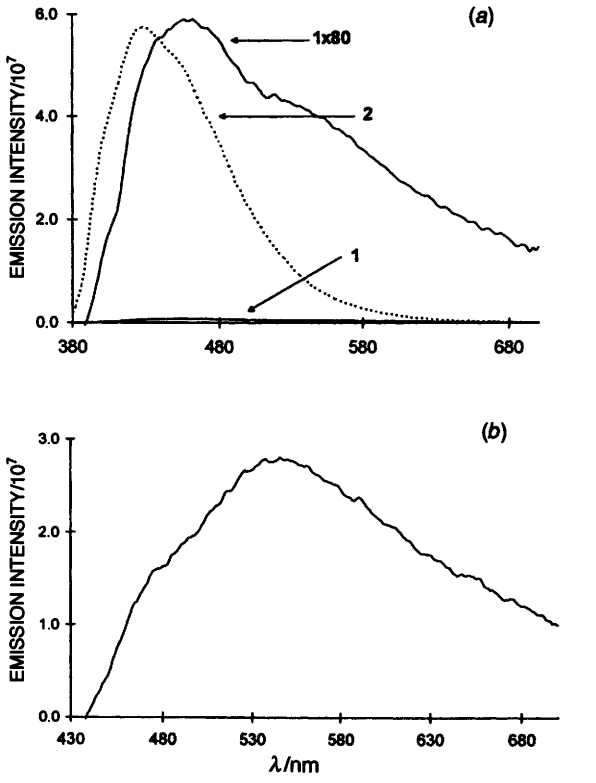

Fig. 2 (*a*) Corrected fluorescence emission spectra of **1** (——) (7.9 × 10^{-6} mol dm^{-3}; λ_{exc} 360 nm; λ_{em} 460 nm, 555 nm) and **2** (· · ·) (7.1 × 10^{-6} mol dm^{-3}; λ_{exc} 359 nm; λ_{em} 428 nm) in 1 mmol dm^{-3} cacodylate buffer, pH 6, 4 mmol dm^{-3} NaCl, 20 °C. (*b*) Excimer spectrum (λ_{em} 555 nm) obtained by difference between the spectra of compounds **1** and **2**.

nitrogens[6,10] to acridine seems negligible according to the pK_a values of the tren linkers mentioned above.

Binding of anionic substrates by the receptor 1

The affinity of the receptor **1** for a wide range of anionic guests has been investigated by means of fluorimetric titrations. The measurements were performed in water at pH 6 in 1 mmol dm^{-3} cacodylate buffer, 4 mmol dm^{-3} NaCl. All the substrates tested did not alter appreciably the absorption properties of **1**, thus the excitation wavelength was fixed at the maximum of absorption (λ_{max} 360 nm). The titrations were carried out by gradual addition of the different substrates to a solution of the receptor **1** and the results were analysed by a non-linear least-squares fitting procedure.

Aliphatic and aromatic polycarboxylates. A typical response to the complexation is that observed for the aliphatic and aromatic polycarboxylates (Table 2, entries 5–12). In all cases, the addition of increasing amounts of the anion induces a strong enhancement of the fluorescence which is accompanied by a blue shift of the monomer-like emission ($\Delta\lambda$ = 32 nm) and the total disappearance of the excimer band [Fig. (3*a*)]. Thus the spectrum at the equilibrium tends to resemble that of the monomeric compound **2** [Fig. 3(*b*)].

These spectral variations are consistent with the formation of complexes in which the acridine rings are prevented from forming an intramolecular excimer. The decrease of the mutual interactions between the three chromophores is indicative of conformational changes of **1** to accommodate the anionic guest which might be more or less included inside the cavity.

The magnitude of the fluorescence increase is illustrated by the relative quantum yield Φ/Φ_0 (Table 2). This effect appears to be mainly related to the structural features of the substrate (chain length, aromatic character and disposition of the functional groups). (*i*) In the aliphatic series a sudden rise of Φ/Φ_0 is observed when the distance between the two carboxylates

Table 2 Maximum emission wavelengths (λ_{em}), relative fluorescence quantum yields (Φ/Φ_0) and calculated stability constants (log K_s) of the complexes of receptor **1** with anionic substrates

Entry	Substrate[a]	λ_{em}/nm	Φ/Φ_0[b]	log K_s[c]
1	acetate^{1-}	460, 555	1	—
2	malonate^{2-}	460, 555	1	—
3	maleate^{2-}	460, 555	1	—
4	succinate^{2-}	460, 555	1	—
5	glutarate^{2-}	428	3.3	2.7
6	adipate^{2-}	428	3.3	3.6
7	pimelate^{2-}	428	3.5	3.2
8	benzoate^{1-}	440	1.2	*d*
9	OP^{2-}	434	2.2	3.5
10	MP^{2-}	428	18.3	4.1
11	TP^{2-}	428	27.4	4.2
12	BTC^{3-}	432	4.1	7.0
13	NDC^{2-}(2,6)	480	4.2	*d*
14	NDS^{2-}(1,5)	480	7.3	*d*
15	AMP^{2-}	443	2.8	3.95
16	ATP^{4-}	434	1.9	7.0
17	UMP^{2-}	460, 555	1.1	*d*
18	UTP^{4-}	434	3.1	4.8
19	p(dA)$_9$	460, 555	1.5	*d*
20	p(dT)$_9$	428	10	*d*
21	HPO_4^{2-}	460, 555	1	—
22	ClO_4^-	460, 555	1	—
23	SCN^-	460, 555	1	—
24	$(CH_3)_2AsO_2^-$	428	2.8	*d*

[a] The anionic substrates were used as sodium or potassium salts: OP^{2-}, orthophthalate; MP^{2-}, metaphthalate; TP^{2-}, terephthalate; BTC^{3-}, benzene-1,3,5-tricarboxylate; NDC^{2-}(2,6), naphthalene-2,6-dicarboxylate; NDC^{2-}(1,8), naphthalene-1,8-dicarboxylate; NDS^{2-}(1,5), naphthalene-1,5-disulfonate; AMP^{2-}, adenosine monophosphate; ATP^{4-}, adenosine triphosphate; p(dA)$_9$, oligodeoxyadenylic acid; p(dT)$_9$, oligodeoxythymidylic acid. [b] The Φ/Φ_0 values were obtained from the ratio of the emission peaks areas of compound **1** in the absence and presence of substrate by extrapolation to 100% complexation on the basis of the stability constants. [c] Standard deviation ±0.2. [d] Not determined, see text; in these cases the Φ_r values were obtained from the ratio of the emission peaks areas of the compound **1** in the presence and absence of substrate at saturation.

increases from 2 to 3 CH_2 groups (entries 4 and 5). Thus a minimum chain length of three methylene units seems to be required to induce a significant increase of the fluorescence of **1**. The formation of less stable complexes or the existence of conformations where the acridine nuclei are not parted from each other might be postulated with the shorter substrates. (*ii*) The remarkably high quantum yields obtained for the aromatic dicarboxylates TP^{2-} and MP^{2-} (entries 10, 11), as compared to the aliphatic derivatives, express a much larger perturbation of the local environment of the acridine nuclei. This might result from the contribution of both steric and hydrophobic factors: a planar aromatic guest would be more appropriate for insertion between two other aromatic units and thus would favour the separation of the chromophores; furthermore the specific π–π interaction occurring between the aromatic rings of the substrate and the acridines of **1** might influence strongly the emission of the complexes. Actually the addition of the terephthalate dianion to the reference monomeric compound **2** results in a significant increase of the emission of **2** (Φ/Φ_0 = 1.5) whereas no spectral perturbation is recorded upon addition of the adipate disodium salt. The strong variation of the signal seems thus to reflect the additive contributions of the π–π interaction and of the high degree of structural complementarity between the substrates and the receptor **1**. (*iii*) The relatively weak effect induced by the binding of orthophthalate (entry 9), in contrast to that of the *meta* and *para* isomers, is consistent with a strong discrimination based on the relative disposition of the two carboxylic functions. (*iv*) The strong enhancement of

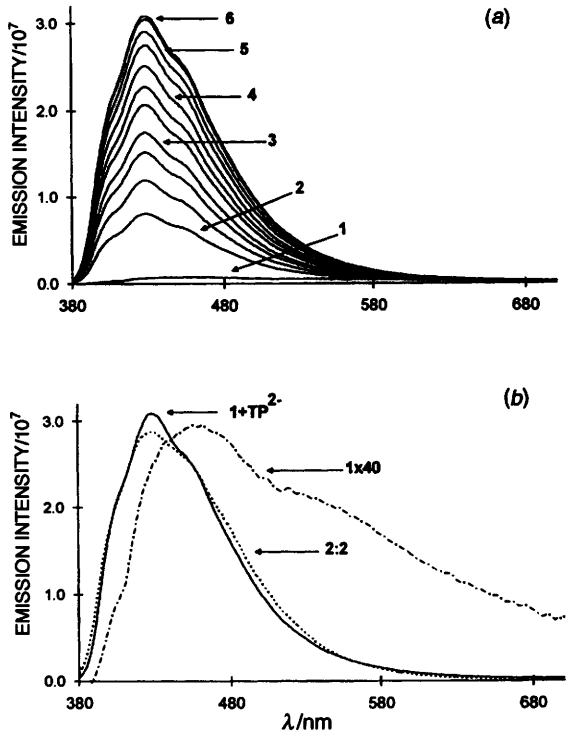

Fig. 3 (*a*) Fluorimetric titration of **1** (7.9 × 10^{-6} mol dm^{-3}; λ_{exc} 360 nm), with terephthalate disodium salt in 1 mmol dm^{-3} cacodylate buffer, [TP^{2-}]/[**1**]: 1: 0, 2: 2, 3: 8.4, 4: 27, 5: 62 and 6: 100. (*b*) Comparison of fluorescence emission spectra of **1** (7.9 × 10^{-6} mol dm^{-3} λ_{exc} 360 nm) free (– · – · –); in the presence of terephthalate disodium salt (——); [TP^{2-}]/[**1**] = 100 and of **2** (· · ·) (7.1 × 10^{-6} mol dm^{-3}; λ_{exc} 359 nm).

the fluorescence of **1** induced in both series by the dianionic species (entries 5–7, 9–11) as compared to the monoanionic compounds (entries 1 and 8) demonstrates the important role played by the electrostatic forces in the complexation. The lower value of Φ/Φ_0 observed for the complex of **1** with the tricarboxylic derivative BTC^{3-} (entry 12) suggests the involvement in this case of intrinsic parameters such as the lower hydrophobicity of the substrate or a different mutual orientation of the transition moments of the chromophores which could contribute to moderate the effect on the fluorescence of the receptor.

The quantitative treatment of the data is in agreement with the formation of complexes of 1 : 1 stoichiometry. Examination of the association constants reveals that in most cases the stability of the complexes is in accordance with the variation of the quantum yield (Table 2). The most stable associations are obtained with the highly charged species (K_S dicarboxylates > K_S monocarboxylates) and with the aromatic guests (K_S aromatic series > K_S aliphatic series). The high affinities of **1** for the multiply charged anionic compounds and for the aromatic substrates emphasize the importance of both hydrophobic and electrostatic phenomena in the molecular recognition properties of such positively charged receptors. Finally the greater stability constant measured for the complex of **1** with adipate as compared to either shorter or longer substrates agrees with the chain length selectivity already mentioned for a tris-biphenyl cryptand.[3b]

Naphthalene-carboxylates and -sulfonates. The complexation of naphthalene-2,6-dicarboxylate (NDC^{2-}, entry 13) induces an increase of the fluorescence of **1** but with a ratio Φ/Φ_0 lower than those observed for the benzenedicarboxylates. Moreover the shape of the spectrum appears quite different; it exhibits a structureless broad band with an emission maximum at 480 nm reaching a limiting value for a ratio [NDC^{2-}]/[**1**] = 2–3 (Fig. 4).

The fluorescence of NDC^{2-} itself (λ_{exc} 293 nm) shows a

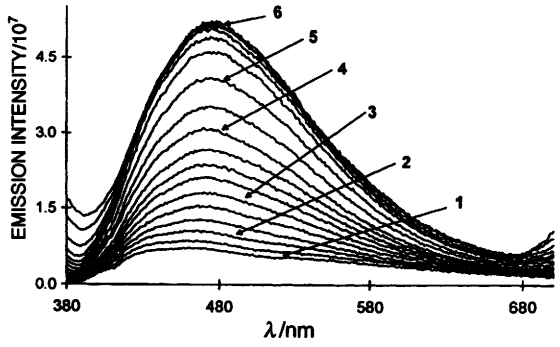

Fig. 4 Fluorimetric titration of **1** (7.9×10^{-6} mol dm^{-3}; λ_{exc} 360 nm), with naphthalene-2,6-dicarboxylate dipotassium salt in 1 mmol dm^{-3} cacodylate buffer, [NDC^{2-}]/[**1**]: 1: 0, 2: 0.2, 3: 0.5, 4: 1, 5: 1.5 and 6: 2.8

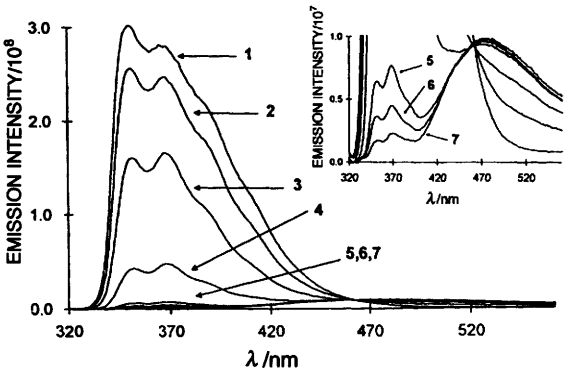

Fig. 5 Fluorimetric titration of NDC^{2-} (2.2×10^{-5} mol dm^{-3}; λ_{exc}, 293 nm), with compound **1** in 1 mmol dm^{-3} cacodylate buffer, [**1**]/[NDC^{2-}]; 1: 0, 2: 0.2, 3: 0.35, 4: 0.5, 5: 0.7, 6: 0.8, 7: 1. Insert: scaled by a factor of 30.

spectacular decrease in intensity (λ_{em} 350 nm to 368 nm) as the concentration of the tris-acridine compound **1** increases (Fig. 5). This quenching is accompanied by the concomitant increase in the fluorescence intensity of **1** at 480 nm (Fig. 5, insert). The contribution of the directly excited tris-acridine cryptand molecules (at 293 nm) to this increase was found to be a small fraction of the overall fluorescence of **1** (Fig. 6). These results might be interpreted in terms of transfer of excitation energy from the naphthalene (donor) to the acridine chromophore (acceptor) as suggested from the overlap of the emission and absorption spectrum of the NDC^{2-} and **1** (Fig. 7). The calculation of the transfer efficiency[11] (Φ_T) through observation of the fluorescence decrease of the NDC^{2-} gives a value close to 1 ($\Phi_T > 0.99$) which tends to demonstrate the high efficiency of the process. However, the low quantum yield of the acridine emission at 480 nm compared to that of the naphthalene and the important overlap of the two emission spectra (as seen in Fig. 5, insert) does not allow a complementary measurement of Φ_T from the enhancement of the fluorescence of the complex in order to check the value determined from the decrease of the donor. Thus, the contribution of other deactivation modes such as the quenching of the NDC^{2-} fluorescence by the acridine cannot be excluded. Finally, agreements between the values of the molar fraction X = [NDC^{2-}]/[**1**] required to reach the equilibrium in both experiments ($X = 2$, Fig. 4; $1/X = 0.5$, Fig. 5; determined from the binding curves, see below) suggest that there is little or no intervention of external free naphthalenic molecules and that the spectral variations rely essentially on the complexation event.

The quantitative analysis of the binding curves reveals some difficulties to fit the experimental data with the 1:1 stoichiometry. Indeed a complementary Job plot experiment measuring the variation in fluorescence intensity of NDC^{2-} as a function of the ratio [NDC^{2-}]/[NDC^{2-} + **1**] at constant [NDC^{2-} + **1**], passes through a maximum at 0.6 and seems to indicate the presence of a certain amount of higher order

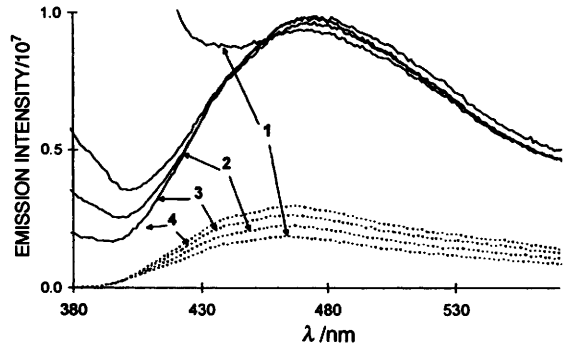

Fig. 6 Comparison of fluorescence emission spectra of **1** (λ_{exc} 293 nm) free (· · ·) and in presence of NDC^{2-} (——) (2.2×10^{-5} mol dm^{-3}), at increasing concentrations of **1**, 1: 11.3×10^{-6} mol dm^{-3}; 2: 14.9×10^{-6} mol dm^{-3}; 3: 18.9×10^{-6} mol dm^{-3} and 4: 2.2×10^{-5} mol dm^{-3}

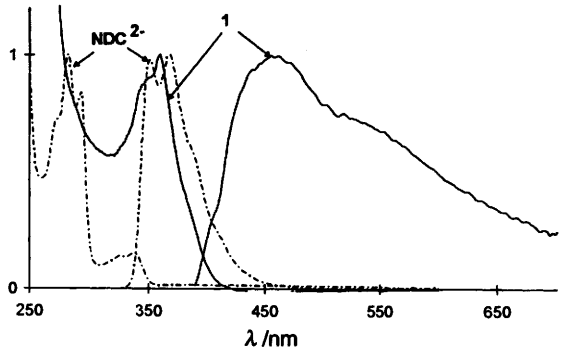

Fig. 7 Absorption and emission spectra of **1** (——) and NDC^{2-} (– · – · –) in 1 mmol dm^{-3} cacodylate buffer; all spectra have been normalized to the same height at the maximum. The molar extinction coefficients at 293 nm (excitation wavelength) are 1.2×10^4 and 8×10^3 dm^3 mol^{-1} cm^{-1} for NDC^{2-} and **1**, respectively.

species, likely those associating two moles of NDC^{2-} with one mole of cryptand. For this reason the stability constant cannot be determined accurately, but considering the Job plot results, the formation of a 1:1 complex with a high stability (log $K_S > 5$) should be dominant.

Similar spectral features have been observed in the binding of **1** with the naphthalene-1,5-disulfonate(NDS^{2-}, entry 14) but with a lower affinity of the two compounds, as 60 equivalents of NDS^{2-} are necessary to saturate the receptor. Moreover, despite the observation of the strong decrease of the fluorescence of the naphthalenic derivative when titrated by **1**, the concomitant increase of the fluorescence of the receptor is close to that of the directly excited molecules, likely to be due to the low proportion of complex formed. The presence of a large amount of free molecules of substrate does not allow the quantitative analysis of the process.

The difficulties encountered in properly interpreting and analysing the experimental results in these cases emphasize the influence of various parameters in the fluorescence variation of **1** through the complexation of the naphthalene derivatives. The superimposed contributions of both conformational changes of **1** and various interactions at the excited state (energy transfer, quenching) between the naphthalene and acridine moieties are likely to the responsible for this, as well as the formation of a mixture of complexes. In conclusion, the inclusive cryptate nature of the complexes cannot be assessed precisely but the probable occurrence of energy transfer reflects the close proximity of the chromophores of both receptor and substrate and thus the major formation of a tight and intimate association.

Nucleotides and oligonucleotides. As expected from previous studies,[4d,e] receptor **1** tightly binds the nucleotides and oligonucleotides, especially those with a high charge density (entries 15–20). In the case of purines, the emission spectra of

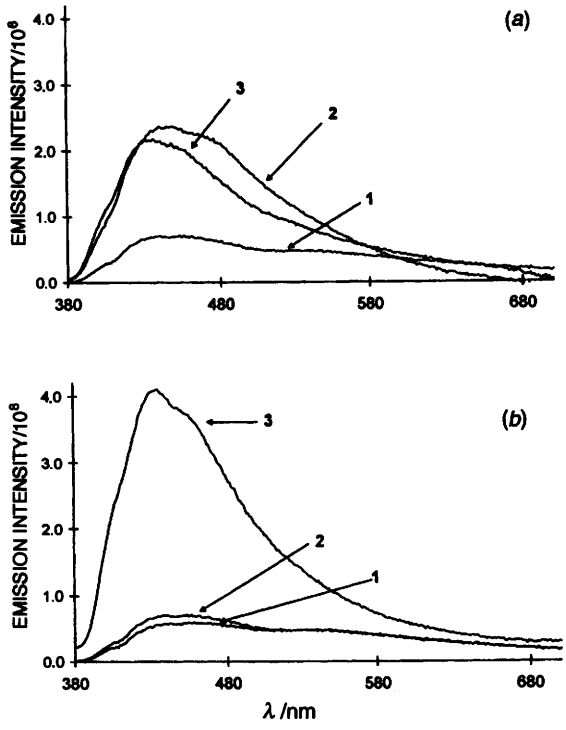

Fig. 8 (*a*) Corrected emission spectra of **1** (7.9×10^{-6} mol dm^{-3}; λ_{exc} 360 nm), in 1 mmol dm^{-3} cacodylate buffer: 1: free; 2, in presence of AMP^{2-}, $[AMP^{2-}]/[\mathbf{1}] = 60$; 3: in presence of ATP^{4-}, $[ATP^{4-}]/[\mathbf{1}] = 1$. (*b*) Corrected emission spectra of **1** (7.9×10^{-6} mol dm^{-3}; λ_{exc} 360 nm), in 1 mmol dm^{-3} cacodylate buffer: 1: free; 2, in presence of UMP^{2-}, $[UMP^{2-}]/[\mathbf{1}] = 100$; 3: in presence of UTP^{4-}, $[UTP^{4-}]/[\mathbf{1}] = 50$.

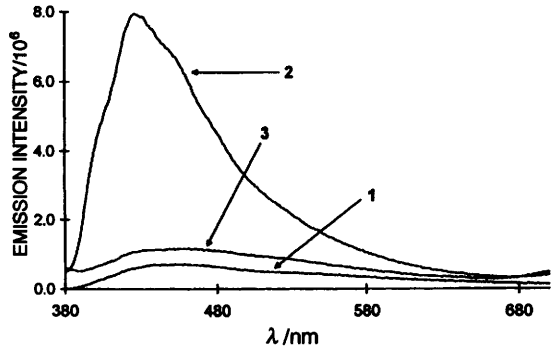

Fig. 9 Corrected emission spectra of **1** (5.5×10^{-6} mol dm^{-3}; λ_{exc} 360 nm), in 1 mmol dm^{-3} cacodylate buffer; 1: free; 2: in presence of $p(dT)_9$, $[p(dT)_9]/[\mathbf{1}] = 0.5$; 3: in presence of $p(dA)_9$, $[p(dA)_9]/[\mathbf{1}] = 4$

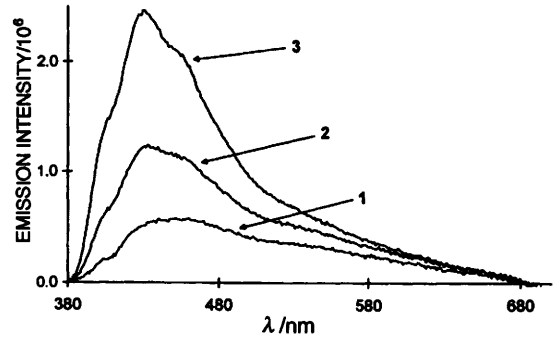

Fig. 10 Corrected fluorescence emission spectra of **1** (7.9×10^{-6} mol dm^{-3}; λ_{exc} 360 nm), at increasing concentrations of cacodylate buffer: 1 = 1 mmol dm^{-3}; 2 = 10 mmol dm^{-3} and 3 = 50 mmol dm^{-3}

the complexes, centred around 430–450 nm, are intermediate in shape between that of the monomer **2** and that of the free receptor **1** [Fig. 8(*a*)]. Purines are known to quench the emission of the acridine chromophores[4e] and the observed fluorescence should be a compromise between two opposite influences; enhancement of the emission due to conformational modifications of **1** and fluorescence quenching resulting from specific interaction between the aromatic rings of the host and the guest. This could explain the moderate enhancement of the quantum yield observed for AMP^{2-} and ATP^{4-} which both form highly stable complexes with **1**. In the case of pyrimidines, the weak perturbation induced by the monophosphate derivative UMP^{2-} is in contrast with the well defined spectrum centred at 434 nm recorded from the complexation of the trinucleotide UTP^{4-} [Fig. (8*b*)]. This again illustrates the determinant influence of the electrostatic factors on the stability of the complexes. Finally, our measurements clearly confirm the stronger complexation of the purine derivatives (K_S, ATP^{4-} > K_S, UTP^{4-}); the purine/pyrimidine selectivity which is commonly observed in the binding of nucleotides in aqueous media,[4d,e] reveals a significant hydrophobic interaction due to a certain degree of stacking between the aromatic moieties of **1** and the nucleic bases.

The interaction of **1** with oligomeric adenylic and thymidylic acids has also been studied (entries 19 and 20) and a great difference in the optical response of the bound receptor is observed depending on the series. A strong increase of the fluorescence of **1** is recorded ($\Phi/\Phi_0 = 10$) upon addition of 0.5 mol equiv. of the oligopyrimidinic sequence $p(dT)_9$ and the spectrum resembles that of the monomer with an almost complete suppression of the excimer band (Fig. 9). In contrast, the binding of the oligopurine $p(dA)_9$ induces only weak spectral variations even at higher molar ratios $\{[p(dA)_9]/[\mathbf{1}] > 4\}$. The titration by $pd(T)_9$ presents two steps: (*i*) at low molar ratios $\{[p(dT)_9]/[\mathbf{1}] < 0.2\}$ the overall shape of the spectrum is not modified but its intensity is significantly enhanced ($\Phi/\Phi_0 = 2$–3); and (*ii*) at higher ratios $\{[p(dT)_9]/[\mathbf{1}] > 0.3\}$ the spectrum changes progressively to a strong peak at 428 nm. The former step should correspond to the association of the two partners essentially driven by the electrostatic energy and the latter to the formation of a complex of well defined structure and stoichiometry with a tighter anchorage of the pyrimidine rings to the acridine units of the receptor. Unfortunately, further addition of oligonucleotide gives rise to precipitation which hinders the determination of the equilibrium constant; however the affinity appears to be very high considering the small amount of nucleotidic material required to obtain a response from the receptor.

Receptor **1** thus appears able to discriminate by its optical properties between a pyrimidic and a purinic sequence. However further experiments are required to properly interpret these interactions, to confirm the selectivity and then to consider if the spectroscopic properties of **1** could be applied to detect homopyrimidine sequences in a single stranded DNA or RNA.

Interaction of receptor 1 with inorganic ions—buffer effects

Owing to the ability of the bis-tren macrocycles to encapsulate inorganic polyatomic anions (N_3^-, ClO_4^-),[3a,c] we examined the influence of various inorganic salts on the fluorescence of the receptor **1**. Most of the species tested did not appreciably perturb the fluorescence spectra of **1** except sodium cacodylate (dimethylarsinate) (entries 21–24). In fact the fluorescence spectrum of **1** at increasing concentration of cacodylate buffer shows a remarkable increase of quantum yield (Fig. 10): in 1 mmol dm^{-3} cacodylate buffer the spectrum is similar in shape and in intensity to the spectrum of **1** at pH 6 in water without buffer, whereas at 10 mmol dm^{-3} and 50 mmol dm^{-3} buffer the signal displays the same features as those observed for the complexes of the receptor with organic substrates, *i.e.* there is an increase of the monomer band and disappearance of the excimer contribution. However, this effect occurs at a range of concentrations much

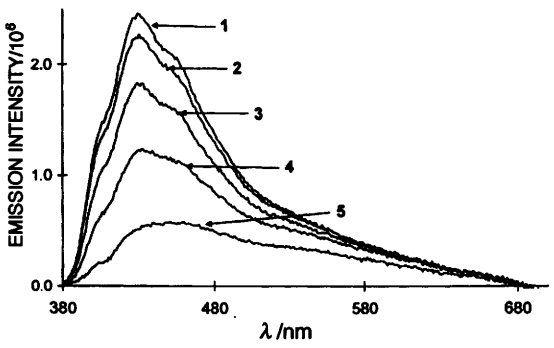

Fig. 11 Corrected emission fluorescence spectra of **1** (7.9×10^{-6} mol dm^{-3}; λ_{exc} 360 nm), in 50 mmol dm^{-3} cacodylate buffer, at increasing concentrations of phosphate dipotassium salt: 1: 0 mmol dm^{-3}; 2: 0.37 mmol dm^{-3}; 3: 1.8 mmol dm^{-3}; 4: 5.4 mmol dm^{-3} and 5: 20 mmol dm^{-3}

higher (10 mmol dm^{-3}–50 mmol dm^{-3}) than that required for the binding of the organic anions (1 μmol dm^{-3}–0.1 mmol dm^{-3}).

Since the emission of the monomer **2** is not affected by the concentration of the cacodylate buffer, this variation may be attributed to the binding of the arsinate ions by the tren moieties, in close proximity to the chromophores. The cacodylate buffer does not perturb the fluorescence of a bis-naphthalene macrocyclic compound[4d] described previously while a similar influence of the arsinate ions has been observed on the emission of related bis-acridine and bis-quinacridine structures.[4e,12] This effect thus seems specific to the acridine ring.

The variation of the fluorescence appears reversible upon gradual addition of a 20 mmol dm^{-3} solution of phosphate dipotassium salt (Fig. 11), the spectrum at equilibrium being the same as that observed for **1** in 1 mmol dm^{-3} cacodylate. This reversibility reflects the displacement of the arsinates by the phosphate ions. Similar competition experiments have been conducted with SCN^- and ClO_4^- and led to the same observations. On the other hand, high concentrations of various buffers (Pipes, Hepes, Trizma) do not induce any significant variation of the fluorescence of **1**. The high concentration of arsinate ions created in the microenvironment of the acridine nuclei by the electrostatic attraction of the polyammonium groups might explain these results. The resulting perturbation of the local polarity could thus be responsible for the variation in the fluorescence intensity of the three chromophores.

Nevertheless, the revival of the fluorescence of the receptor **1** at high concentrations of cacodylate buffer could be used to evaluate, by means of displacement experiments, the affinity of **1** for various inorganic anions which are intrinsically without effect on the fluorescence of the receptor.

Conclusions

The macrobicyclic receptor **1** has the potential to give a highly sensitive light-emissive response on complexation of a large range of organic anions. The restoration of the fluorescence of **1** allows the determination of the stability of the complexes, which gives information about the factors involved in the strength of the association (electrostatic attraction, hydrophobic forces, structural complementarity). On the other hand, the magnitude of the emission increase, which is really remarkable in some cases, appears to rely largely on the specific π–π interaction between the aromatic moieties of the substrate and the acridine units of **1**.

In summary the binding selectivities of cryptand **1**, combined with its photophysical properties, offer attractive features for its use as a fluorescent sensor for the detection of anions and polyanions in water especially in the case of biological targets such as nucleic acids.

Experimental

All commercially available chemicals employed were reagent grade and used without further purification. Proton NMR spectra were recorded on a Bruker AC 200 (200 MHz) spectrometer and UV spectra were obtained using a Beckmann model DU 460 spectrophotometer. The microanalyses were performed at the Service Regional de Microanalyse de l'Université Pierre et Marie Curie (Paris).

Preparation of the macrobicyclic cryptand 1

A solution of tris(2-aminoethyl)amine (tren) (165.5 mg, 1.1 mmol) in CH_2Cl_2–MeOH, 1:1 (30 ml) was added dropwise, at room temperature and under N_2, to a well stirred solution of acridine-2,7-dicarbaldehyde (400 mg, 1.7 mmol) in CH_2Cl_2–MeOH, 1:1 (300 ml). The mixture was stirred at room temperature for 15 h and then slowly evaporated until a precipitate was formed. It was collected, washed with diethyl ether and dried under vacuum to yield a yellow powder (405 mg) corresponding to the hexaimino intermediate. The crude extract (200 mg, 0.2 mmol) was then redissolved in CH_2Cl_2–MeOH, 2:1 (120 ml) and $NaBH_4$ (86 mg, 2.3 mmol) was added to this solution cooled at 0 °C. After stirring for 4 h, the solvents were evaporated and the residue was dissolved in water (20 ml) and extracted by CH_2Cl_2–MeOH, 9:1. The organic phase was dried and evaporated. The residue was recrystallized twice in 1 mol dm^{-3} HCl–EtOH mixture to yield **1-11H**$^+$ as a yellow powder (150 mg, 40%); $\delta_H(D_2O$, pH 3.3) 2.84 (s, 12 H), 3.34 (s, 12 H), 4.3 (s, 12 H), 8.02 (s, 6 H), 8.15 (dd, 12 H), 9.09 (s, 3 H) (Calc. for $C_{53}H_{74}N_{11}Cl_{11}$ (**1-11HCl**): C, 52.53; H, 5.72; N, 11.82. Found: C, 52.34; H, 5.75; N, 11.70).

Fluorescence measurements

The fluorescence measurements were performed with a Fluoromax apparatus (Spex) equipped with an Hamamatsu R928 photomultiplier (PM), using a thermostatted cell holder. The data have been corrected for the response of the PM. The fluorescence quantum yields (Φ_F) were determined by comparison with quinine sulfate in 0.05 mol dm^{-3} sulfuric acid ($\Phi_F = 0.55$) and anthracene in ethanol ($\Phi_F = 0.27$). The titration experiments were performed at 20 °C as follows. To an aqueous solution of **1** (7.9×10^{-6} mol dm^{-3}) in 1 mmol dm^{-3} cacodylate buffer (4 mmol dm^{-3} NaCl), were added aliquots of a mixture of the substrate diluted in an aqueous solution of **1** (7.9×10^{-6} mol dm^{-3}); in this manner the concentration of the receptor was kept constant while the concentration of the substrate varied. The data were analysed by using a non-linear least-squares fitting procedure.

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